Título: GENE-ACTIVATED CRYOGELS FOR CARTILAGE REPAIR

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Resumen: The increase in life expectancy associated with developments in the field of medicine has prompted the prevalence of degenerative diseases related to aging. Specifically, articular cartilage is a tissue with a very limited ability to self-repair upon injury due to its avascular and aneural nature. Hence, osteoarthritis (OA) represents the most common cause of long-term pain and physical disability in developed countries. However, none of the current therapeutic options has been able to completely restore the function of hyaline cartilage, generally leading to the formation of fibrocartilage.

In this context, gene therapy has emerged as a promising alternative to treat articular cartilage injuries by transferring therapeutic genes into the lesion site. Non-viral vectors represent the safest tools to accomplish this aim as they avoid the main drawbacks of viral carriers, including the risk of eliciting insertional mutagenesis or immune responses in the host. Nonetheless, the existence of several extracellular and intracellular barriers considerably reduced their efficiency compared to their viral counterparts. Noteworthy, the design of gene-activated matrices (GAMs) may help to overcome these issues by promoting a controlled delivery of the candidate genes into the cellular microenvironment.

This dissertation focuses on the production of a gene-activated cryogel (G-HACG) based on a combination of non-viral vectors, a shape memory hyaluronic acid-based cryogel (HACG), and a source of primary

mesenchymal stem cells (MSCs). Developed cryogel systems showed a macroporous structure mimicking the composition of the cartilage extracellular matrix with great biocompatibility and promoting cell proliferation. Various non-viral gene delivery systems based on niosomes were produced and their composition was optimized to obtain high levels of transfection in MSCs with a reduced cytotoxicity. Best niosome formulations were tested as carriers of a plasmid encoding for the transcription factor SOX9 (nioplexes) to promote MSC chondrogenesis. After refining their composition, nioplexes were first incorporated into the cryogels and their release profile and bioactivity profile were monitored. Lastly, MSCs were incorporated into these systems to produce the G-HACG. Effective chondrogenesis of MSCs inside G-HACG was confirmed, showing a reduced expression of fibrocartilage and hypertrophic markers. A similar trend was observed when administrating G-HACG in an ex vivo model of chondral defect, highlighting the potential of the developed systems for restoring cartilage extracellular matrix.